

## EGGS

The U.S. Food and Drug Administration (FDA) has classified hydrogen peroxide as a low regulatory priority drug when used at concentrations of up to 500 mg/L to control fungal infections on fish and their eggs. Hydrogen peroxide appears to have the potential to be a broad spectrum fish therapeutant. However, FDA requires further research and data submissions before granting a New Animal Drug Approval for additional uses for hydrogen peroxide. Additional studies are being conducted to gain FDA approvals for using hydrogen peroxide to control fungal and bacterial infections and parasitic infestations on freshwater fish. Funding for this research was provided by the International Association of Fish and Wildlife Agencies.

## OBJECTIVES

- To evaluate the efficacy of hydrogen peroxide therapy regimens for the control of fungal and bacterial gill disease infections and parasitic infestations on cultured fish.
- To provide data for a New Animal Drug Application that may expand the use of hydrogen peroxide to include the treatment of fungal and bacterial infections and parasitic infestations of fresh water fish.

## METHODS

Test Parameters	Diseases		
	Bacterial Gill Disease	Fungus (Eggs)	Parasites
Species	Brown and Rainbow Trout	Cold-, Cool, and Warmwater Fish <sup>1</sup>	Rainbow Trout
Test System	Tanks	Egg Jars/Heath Incubator	Tanks
Concentrations	56, 110, 230 mg/L	250-6,000 mg/L	170, 280, 560 mg/L
Exposure Time	30 or 60 min	15 min	30 min
Treatment Sequence	Every Other Day	Daily	Every Other Day
Number of Treatments	3	Varies – Time to Hatch	3
Evaluation of Efficacy	Fish Mortality	Percent Hatch	Change in Numbers

<sup>1</sup> Rainbow Trout, Walleye, White Sucker, Northern Pike, Lake Sturgeon, and Channel Catfish



Aquaculture is important for sport fishing, human consumption, and re-establishment of endangered native fish populations.



Chemicals are essential in intensive fish culture systems.

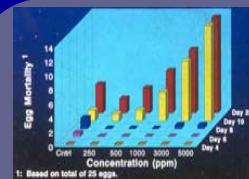


Figure 3. Cumulative mortalities of rainbow trout eggs treated with hydrogen peroxide for 15 minutes over a 22-day hatch period.



Miniature egg jar incubation system contained a headbox, egg jars, and aquaria.

## Results

### Sensitive Stage - Figure 3

- Rainbow trout eggs exhibited a sensitive stage between 70 - 140 Daily Temperature Unit (DTU; °C) where concentrations  $\geq 1,000$  mg/L increased mortality in comparison to the control group.
- The toxicity was highest in the 3,000 and 5,000 mg/L treatment group.
- This sensitive egg stage was not observed in the cool- and warmwater fish species treated with hydrogen peroxide, however, culturists should be aware this sensitive stage may exist and caution should be used when treating any fish species.

## EGGS

Table 1. Percent egg hatch of 5 species of fish treated daily with hydrogen peroxide concentrations of either 500, 1,000, 3,000, or 6,000 mg/L for 15 min until hatch complete.

Fish Species	Treatment Concentration (mg/L)			
	Control	1,000	3,000	6,000
Northern Pike	32	37	34	Not tested
Walleye	0	77	61	5
White Sucker	15	61	42	0
Lake Sturgeon	51	57	61	40
Channel Catfish	19	78	68	0

## Results

### 5 Species - Table 1

- All control egg species became infected with fungus, except northern pike eggs.
- Treatment groups were free of fungal infections.
- The 1,000 mg/L was generally the most efficacious in increasing the percent egg hatch.
- The 6,000 mg/L hydrogen peroxide treatment was toxic to the eggs.

## PARASITES



Hydrogen peroxide concentrations were verified by a titration method.

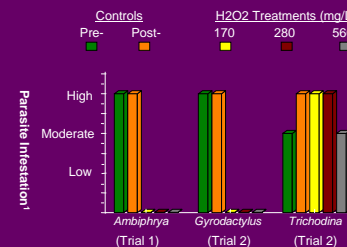


Figure 4. Rainbow trout infested with parasites (*Ambiphyra*, *Gyrodactylus*, or *Trichodina*) were treated with hydrogen peroxide concentrations of 0, 170, 280, and 560 mg/L for 30 minutes once per day every other day on three occasions.

## Results

- In trial 1, all hydrogen peroxide treatment regimens eliminated *Ambiphyra* (protozoan) while the control fish remained highly infested with the parasite.
- In trial 2, hydrogen peroxide treatments eliminated *Gyrodactylus* (digenetic trematode) from all test fish, while the control fish remained infested with the parasite.
- In trial 2, hydrogen peroxide treatments were not effective in the elimination of *Trichodina* (protozoan).
- Fish exposed at 170 mg/L behaved similar to the control group, while fish in the 280 and 560 mg/L treatments were observed surfacing, jumping and opercular movement was reduced.

## CONCLUSIONS

- In cold-, cool-, and warmwater fish species, hydrogen peroxide concentrations of 500 and 1,000 mg/L generally increased the percent egg hatch in comparison to untreated controls. Hydrogen peroxide treatments were effective in controlling the spread of fungus to healthy eggs.
- In some coldwater fish species, there is a sensitive developmental egg stage where toxicity to hydrogen peroxide increased between 70 - 140 DTU (°C).
- Hydrogen peroxide treatment regimens of 56 and 110 mg/L applied for 60 minutes or 30 minute exposures of 56, 110, and 230 mg/L reduced mortalities in coldwater fish species infected with bacterial gill disease.
- Hydrogen peroxide treatment regimens of 170 - 280 mg/L applied for 30 minutes were effective in reducing or eliminating certain species of protozoan and digenetic trematode parasites.
- Variables such as stage of disease development, water chemistry, temperature, fish life stage, species, and health of the fish must be considered before selecting a specific treatment concentration and exposure time.
- A preliminary bioassay should be conducted on a few fish to determine the safety of the proposed treatment prior to treatment of an entire group of fish.

## BACTERIAL GILL DISEASE



Portable testing system used to evaluate the efficacy of hydrogen peroxide treatments.

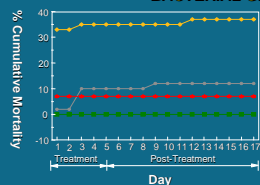


Figure 1. Percent cumulative mortality of brown trout treated with hydrogen peroxide concentrations of 0, 56, 110, and 230 mg/L for 60 minutes once per day every other day on three occasions.

## Results

### Brown Trout - Figure 1

- Brown trout in the 56 mg/L treatment behaved similar to the controls, while the fish in the 110 and 230 mg/L treatment groups exhibited signs of stress.
- When comparing mortality in the control group to the treatment groups, lower mortalities occurred in the 56 and 110 mg/L while a significantly higher mortality rate was observed in the 230 mg/L treatment group.
- Post treatment (12-13 days after last treatment) observations indicated the gills of treated fish appeared normal, while the control fish gills exhibited hyperplasia.

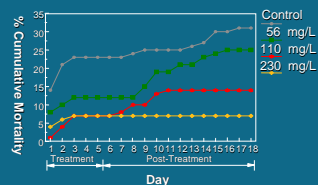


Figure 2. Percent cumulative mortality of rainbow trout treated with hydrogen peroxide concentrations of 0, 56, 110, and 230 mg/L for 30 minutes once per day every other day on three occasions.

## Results

### Rainbow Trout - Figure 2

- Rainbow trout in the 56 and 110 mg/L treatments behaved similar to the controls, while fish in the 230 mg/L treatment group showed signs of stress.
- Percent mortality was lower in all treatment groups than in the untreated controls.
- Post treatment (12-13 days after last treatment) observations indicated the gills of treated fish appeared normal, while the control fish gills exhibited hyperplasia.



Test system was supplied with water from the raceway containing the diseased fish.